Citation:

Shuaibi AM, House JD, Sevenhuysen GP. Folate status of young Canadian women after folic acid fortification of grain products. *J Am Diet Assoc.* 2008 Dec; 108 (12): 2,090-2,094.

PubMed ID: 19027414

Study Design:

Cross-sectional

Class:

D - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEGATIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

The purpose was to determine folate status and the contribution to total folate intake from natural food folates, folic acid added to food and folic acid from supplements in women of childbearing age from a convenience sample from one Canadian province, Manitoba, in the post-folic acid fortification era.

Inclusion Criteria:

Healthy women aged 17 to 25 years from the University of Manitoba and women who were taking oral contraceptives or folic acid supplements.

Exclusion Criteria:

Women were excluded if they were currently pregnant, had a pregnancy lasting more than 20 weeks in the year before the scheduled clinic visit, were lactating and or used medications known to interfere with folate metabolism.

Description of Study Protocol:

Recruitment

University of Manitoba campus-wide advertisement.

Design

Cross-sectional study.

Dietary Intake/Dietary Assessment Methodology

Folate intake was assessed by the Food Choice Map by trained interviewers. The Food Choice Map nutrient analysis program was designed for collecting data on quality, quantity and preparation of foods and beverages in a standardized way. The Food Choice Map–nutrient analysis program calculations are made with food composition data from 1,462 food items that represent most of the foods reported by this population. Adjustments were made to correct for the bioavailability of folic acid. Food sources and contributors for folate were determined by grouping food items. Twenty-four food groups and subgroups were selected in this attempt. The total amount of folate in each of the selected groups or subgroups was estimated for each participant then divided by the total folate intake from all foods. Foods were ranked by percentage contribution to dietary folate intake.

Blinding Used

Not indicated.

Intervention

Not applicable.

Statistical Analysis

- Biomarker, nutrient and food intake data were analyzed by Number Cruncher Statistical Software (2000 edition, 1999, Kaysville, UT)
- Mean, 10th, 50th and 90th centile values were determined for serum and red blood cell folate and total homocysteine
- Normality of the data was checked by using the Kolmogorov-Smirnov test. Log transformations were used to normalize skewed data. To assess for differences in vitamin intake between supplement users and non-users independent sample T-tests were used. A probability level of 5% was chosen to reflect the level of significance.

Data Collection Summary:

Timing of Measurements

Fasting blood samples measurements were take once after a 12-hour fast.

Dependent Variables

- Total folate concentration from ascorbic acid-stabilized serum with a competitive protein binding assay (Quantaphase Folate/B_{12} Radioassay, Bio-Rad Laboratories, Mississauga, Ontario, Canada). [Negative folate balance equals serum folate concentration of less than 3.1 ng/ml (7nmol/L). Adequate red blood cell folate status equals values greater than or equal to 134.2ng/ml (304nmol/L)].
- Total homocysteine from plasma obtained from ethylenediaminetetraacetic acid-treated blood samples according to the reverse phase–high-performance liquid chromatography method of Araki and Sako, with modifications as suggested by Gilfix and colleagues. (Hyperhomocysteinemic was a total homocysteine concentration greater than 12 micromoles/L).

Independent Variables

Total folate intake from:

- Natural food folates
- Folic acid added to food
- Folic acid from supplements.

Control Variables

None stated.

Description of Actual Data Sample:

- *Initial N:* 95 women
- *Attrition:* 0%
- *Mean age:* 20.5 years (range 17-24 years)
- *Ethnicity:* Not described
- *Other relevant demographics:* None were described
- *Anthropometrics:* None were described
- *Location:* The women were from the University of Manitoba, Canada. Subject characteristics were described in a previous publication: Shuaibi AM, Sevenhuysen GP, House JD. Validation of a food choice map with a three-day food record and serum values to assess folate and vitamin B_{12} intake in college-aged women. *J Am Diet Assoc.* 2008; 108: 2,041-2,050.

Summary of Results:

The mean intakes of folate, as natural food folate, dietary folic acid and folic acid from supplements and Dietary Folate Equivalents (DFEs) of the 95 women, as well as the difference in the intakes between supplement users and non-users, are shown in Table 1.

Sources of folate were determined and the dietary folate equivalents were determined. Table 2.

Table 1. Intakes of natural folate, folic acid from food, folic acid from food and from supplements.

Daily intake mcg per day	Supplement Users and Non-users (N=95)	Supplement Non-users[a] (N=70)	Supplement Users (N=25)	Supplement Users and Non-users (N=95)	Supplement Non-users [a] (N=70)	Supplement Users (N=25)	Supplement Users and Non-users (N=95)	Supplement Non-users[a] (N=70)	Supplement Users (N=25)
	Mean±SD[b]	Median	Range	Mean±SD	Median	Range	Mean±SD	Median	Range
Natural folate from food	314.4±134.3	294.5	84.1-875.5	317.9±135.6	290.6	124.4+875.5	304.3±132.8	314.4	84.2-662.4
Folic acid from food	95.7±63.9	83.1	4.1-311.6	94.9±57.8	86.34	4.1-311.6	97.9±79.8	65.4	16.4-302.2
Folic acid from supplements	94.9±189.3	0	0-1,000	0	0	0	360.8±201.0	400.0	43-1,000
Total DFEs[c]	645.7±368.4	505.4	194.8-2,277.1	486.4±170.0	447.0	194.8-1,215.2	1,092.0±408.2[z]	963.5	458.5-2,277.1

[a] Means compared using independent T-test between supplement non-users and users

[b] SD=standard deviation

[c] DFE=dietary folate equivalent.

[yz] Values with different superscripts (y,z) in row differ significantly

Table 2. Mean, median and range for serum folate.

Variable	Serum folate (nmol/L)	Red blood cell folate (nmol/L)	Plasma total homocysteine (micromol/L)
Mean±standard deviation	14.6±3.5	312.0±137.2	5.3±1.8
Median	14.1	286.8	5.0
Range (10th and 90th percentile)	10.0 to 19.3ng/ml (33.0±7.9,32.0 and 22.6 to 43.8nmol/L)	180.5 to 462.9ng/ml (707±311, 650 and 409 to 1,049nmol/L)	3.5 to 7.5mol/L

Other Findings

1. Folate status was 2.3 times higher than the level deemed acceptable for all women
2. 14% of women had a red blood cell folate concentration greater 400ng/ml (906nmol/L), which is the value associated with very-low neural tube defect risk
3. Mean daily folate intake from food, fortified foods and supplements was 645.7± 368.4mcg DFE per day
4. The contribution of folic acid from supplements to total folate intake, when calculated with the use of the bioavailability correction factor for supplemental folic acid, was 51%. The dietary folate intakes of supplement users from food alone were similar to those of non-users, a finding reported by others.
5. With the use of folic acid-containing supplements and the 1998 folic acid fortification program, 79% and 91% of women in this study met the Recommended Dietary Allowance and Estimated Average Requirement for folate.
6. Only 17% of study participants met the special recommendation for women capable of becoming pregnant (400mcg folic acid per day). No one met this recommendation unless they used folic acid supplements.
7. One percent of participants exceeded the Upper Limit for folate intake and this was due to use of supplements containing 1,000mcg of folic acid
8. The mean increase in folic acid consumption due to fortification reported in our study was 96mcg per day, which suggest that the fortification program is reaching women of reproductive age
9. Vegetables contributed in total 17.7% of the folate consumed by these women. The top folate contributors were breads and bakery products, vegetables, juices, pasta, rice and ready-to-eat cereals.
10. 4.2% of women were classified as hyperhomocysteinemia, which was defined as greater than 12.0micromoles/L
11. No woman was found to have compromised serum folate status.

Author Conclusion:

Data suggest that women of childbearing age are achieving positive folate status in the post-fortification era, but it may not be sufficient to achieve red blood cell folate concentrations associated with a significant reduction in neural tube defect risk.

Reviewer Comments:

It is unclear how many women they initially enrolled in their study.

Limitations noted by the authors

1. *Data may not truly reflect the folate intakes of all women of childbearing age since the data was taken from a convenience sample of women attending a post-secondary educational institution in one Canadian province (Manitoba)*
2. *Lack of a pre-folic acid fortification control group and comparisons of serum and red blood cell folate results obtained from different laboratories and methods may not be directly comparable*
3. *The potential exists for existing nutrient databases to underrepresent the amount of folic acid in fortified foods due to overfortification.*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions

1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes

1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	???
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	No
2.4.	Were the subjects/patients a representative sample of the relevant population?	???
3.	**Were study groups comparable?**	N/A
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	N/A
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	N/A
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	N/A
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	???
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	???
4.1.	Were follow-up methods described and the same for all groups?	N/A
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	N/A
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	???
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N/A
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	Yes
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	???
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	N/A
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	???
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	N/A
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	N/A
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes

	7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
	7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	N/A
	7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
	7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
	7.6.	Were other factors accounted for (measured) that could affect outcomes?	No
	7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**		Yes
	8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
	8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
	8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
	8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
	8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	No
	8.6.	Was clinical significance as well as statistical significance reported?	Yes
	8.7.	If negative findings, was a power calculation reported to address type 2 error?	No
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**		Yes
	9.1.	Is there a discussion of findings?	Yes
	9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**		Yes
	10.1.	Were sources of funding and investigators' affiliations described?	Yes
	10.2.	Was the study free from apparent conflict of interest?	Yes